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Chemical Fate of a Metamorphic Inducer in Larvae-like Buds of the Cnidarian *Cassiopea andromeda*

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Abstract. Larvae-like vegetative buds of the scyphozoan *Cassiopea andromeda* metamorphose into polyps in the presence of oligopeptides that have a well-defined primary structure. Buds were incubated with the hexapeptide ^{14}C-dansyl-GPGGPA, a representative inducer. Autoradiography of longitudinal sections of these buds revealed rapid internalization of peptide by the buds. Silver grain density was highest in the pre-pedal disc region (or aboral knob) of metamorphosing buds. Larvae and buds sporadically explore their habitat with this aboral knob, searching for a suitable solid substrate to which irreversible attachment will be made. Buds were incubated for 3, 8, or 16 h with ^{14}C-dansyl-GPGGPA, then homogenized and the supernatants analyzed to determine the chemical fate of the inducer. The signal molecule was shown to be partly degraded to ^{14}C-dansyl-GP, partly to ^{14}C-dansyl-G, and in part still present in its original structure. These cleavage products were also found in the surrounding medium after an incubation time of 8 h with ^{14}C-dansyl-GPGGPA, but did not induce metamorphosis. This study suggests that exposure of metamorphosis-inducing peptides to buds of *Cassiopea andromeda* results in signal termination.

Introduction

Studies dealing with chemical inducers of metamorphosis of marine invertebrate larvae often focus on their signal transduction systems. Such research has been carried out with the scyphozoan *Cassiopea* spp. In *Cassiopea*, settlement of vegetative buds and larvae and dramatic morphogenetic transition to a primary polyp can be observed in less than 24 h by exposing the animals to oligopeptides of a well-defined primary structure (Fitt and Hofmann, 1985; Fitt *et al.*, 1987; Hofmann and Brand, 1987; Fleck and Hofmann, 1990; Fleck and Bischoff, 1993; Hofmann *et al.*, 1996). Substitution of the amino terminals of metamorphosis-triggering peptides with ligands of increasing hydrophobic character enhances the biological activity, whereas carboxyterminal blockade inactivates it (Hofmann and Brand, 1987; Fleck and Hofmann, 1990; Fleck and Bischoff, 1993; Hofmann *et al.*, 1996).

Biologically active peptides for *Cassiopea* spp. can be easily marked by inserting a radioactively labeled group. Coupling of ^{14}C-dansyl chloride to the amino terminus of the hexapeptide GPGGPA results not only in a labeled inducer molecule but also in a highly efficient trigger of metamorphosis in buds of *Cassiopea andromeda* (Fleck and Hofmann, 1995).

Using this molecule in studies *in vivo*, Fleck and Hofmann (1995) demonstrated specific and saturable binding to buds, suggesting that peptide-induced metamorphosis is receptor mediated. Bioactive peptides are proposed to bind primarily to cell membrane receptors located in the pre-pedal disc region (aboral knob) (Fleck, 1994; Arthecker, 1995; Hofmann and Hellmann, 1995). Swimming with this morphological structure facing forward, buds and larvae explore their habitat in search of a suitable substrate and eventually attach. The natural signal for metamorphic induction of the hydroid *Hydractinia echinata* is also recognized at the aboral end of the larvae (Schwoerer-Böhning *et al.*, 1990). Although the possibility that peptidic cues have to interact with intracellular receptors in order to trigger metamorphosis of *Cassiopea* spp. cannot be excluded, none of the currently available

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Abbreviations: ABS, seawater containing antibiotics; PKC, protein kinase C; TLC, thin layer chromatography.

data favor such a signal transduction mechanism. However, it remains unknown on what part of buds or larvae and in what tissue layers the receptors for the inducers are located.

Tumor-promoting phorbol esters, which are known to activate protein kinase C (PKC, see review by Hug and Sarre, 1993), are another class of biologically active compounds in *Cassiopea* spp. (Bischoff *et al.*, 1991; Fleck and Bischoff, 1993). Studies conducted with phorbol esters, biologically active peptides, and PKC inhibitors indicated that PKC is an important part of the signal transduction pathway in induction of metamorphosis (Bischoff *et al.*, 1991; Fleck and Bischoff, 1993; Fleck, in press). This enzyme has also been shown to be involved in the initiation of metamorphosis in other cnidarian larvae (Freeman and Ridgway, 1990; Hassel *et al.*, 1996; Henning *et al.*, 1996; Walther *et al.*, 1996, Fleck, 1997).

The chemical fate of the metamorphic inducer—after it has produced its biological effect—is a critical matter for signal transduction systems (Carr *et al.*, 1990). Carr *et al.* (1990) reported that chemoactive agents in various organisms are chemically changed by degradative enzymes to yield products that can be either more or less stimulatory than the original compound.

Reports on changes in the chemical structure of the biologically active compounds during interaction with larvae are rare, since even the chemical structure of most natural inducers is not known (see review by Pawlik, 1992). Some inducers are compounds whose chemical fate is difficult to monitor because the molecules are difficult to label; others are of such a simple chemical structure that no further change is to be expected (see review by Pawlik, 1992). This issue is also unexplored for scyphozoans, *i.e.*, it remains unresolved if the inducer molecule is inactivated by either being taken up by larvae or buds of *Cassiopea* or being chemically modified outside of or within larvae or buds.

This paper shows uptake of radioactively labeled peptide and chemical alteration of the signal molecule ^{14}C-dansyl-GPGGPA when exposed to buds of *Cassiopea andromeda* in order to induce metamorphosis. Autoradiographical techniques were used to determine the location—area and tissue—of the receptors for the inducer.

Materials and Methods

Animals

Vegetative buds of *Cassiopea andromeda* were collected from a culture of budding polyps kept in the laboratory at a constant temperature of 23°C in North Sea water. The buds were washed in seawater containing antibiotics (ABS: 100 mg penicillin, 100 mg neomycin, and 130 mg streptomycin dissolved in 1 l seawater) before being used in the assays.

Preparation of ^{14}C-dansyl-GPGGPA

Synthesis of the metamorphosis-inducing oligopeptide ^{14}C-dansyl-GPGGPA is described elsewhere (Fleck and Hofmann, 1995). The total amount of product after purification and lyophilization was about 585 μg (1.9 MBq).

Preparation of bud sections for autoradiographic analysis

Solutions of the inducer peptide were prepared in two glass dishes each containing ^{14}C-dansyl-GPGGPA dissolved in 1 ml ABS in a final concentration of 5.3×10^{-5} mol/l (114 KBq). Forty buds of *Cassiopea andromeda* were incubated in each of the two dishes. A few buds (3–4) were removed after 10, 20, 30, 60, and 120 min and every 2 h thereafter up to a total incubation time of 32 h. The buds were subsequently transferred into 2.5% glutaraldehyde in 0.1 *M* cacodyle buffer and fixed overnight. This procedure was followed by one wash in 0.1 *M* cacodyle buffer and one wash in distilled water, and then by successive rinses in increasing concentrations of ethanol. Embedding was carried out in Epon for 3 days at a temperature of 60°C. The buds were sectioned (1 μm) with an ultramicrotome (Om U3, Reichert). The sections were transferred to slides previously coated with chrome alum gelatin. The slides were covered with stripping film AR 10 (Kodak) in red light and then kept at −20°C for 3 months in darkness. At the end of the exposure, the filmed slides were developed in D19b developer (Kodak) for 5 min, fixed, and dried with cold air from a hair drier. The sections were then stained for 25 min with a solution composed of the following components: 50 ml distilled water, 1.5 ml 0.2 mol/l sodium hydrogen phosphate containing 0.1 mol/l citric acid (pH 7.2), 1.5 ml methanol, and 5 ml Giemsas-solution. After rinsing the slides with distilled water and drying, the specimens were analyzed with a BH-2 light microscope (Olympus).

Silver grain density in the ectoderm and endoderm of the pedal disc, stalk, and presumptive head was determined directly by microscopy (magnification 1000×). Label was counted in five longitudinal sections through the center of buds that had been incubated for either 10 or 30 min in ABS containing ^{14}C-dansyl-GPGGPA. Whole sections were photographed at a magnification of 200×. The number of grains was related to the area (in square centimeters) of the different segments of the developing polyp (pedal disc, stalk, presumptive head). The means of the number of labels of the five sections of each of the three morphological parts were analyzed by analysis of variance (ANOVA): 2-way ANOVAs were performed for the 10-min and 30-min incubation, with segment and tissue (ectoderm, endoderm) as factors, and another 2-way ANOVA was carried out with incubation time and segment as the factors. The Student-Newman-Keuls method

was used for all pairwise multiple comparison procedures ($P < 0.05$).

Determination of the chemical fate of ^{14}C-dansyl-GPGGPA

One thousand buds of *C. andromeda* were incubated in small glass dishes containing ^{14}C-dansyl-GPGGPA dissolved in ABS to make a final concentration of 9×10^{-6} mol/l (17.8 KBq). Inducer peptide at this concentration effects metamorphosis of 100% of buds within 24 h (Fleck and Hofmann, 1995). Incubations were terminated after 3, 8, or 16 h. The animals were washed four times with 5 ml ABS, either in PD 10 columns (Pharmacia) or, if irreversibly attached, in the glass dishes themselves. The buds were homogenized in 1 ml 0.1 *M* HCl by intensive flushing with a glass pipette. The homogenate was transferred to an Eppendorf tube and centrifuged for 5 min at 24000 × *g*. The supernatant, containing cytosol and very small cellular particles, and the pellet, containing cell fragments, were separated. The pellet was completely dissolved in 1 ml 1.35 *M* NaOH overnight. The radioactivity of 100 μl of the supernatant, and of the dissolved pellet, was determined in 2 ml Quickszint 212 (Zinsser) in plastic vials in a Rack Beta liquid scintillation counter (LKB). Both the remaining supernatant (900 μl 0.1 *M* HCl) and pellet (900 μl 1.35 *M* NaOH) were desalted and separated in Mobicol mini columns (Biometra) containing about 300 μl of the reversed phase gel Octyl Si = 100 (Serva). After application of the sample, the gel was washed sequentially with 2 ml distilled water, and then 30, 50, 70, and 100% methanol. Radioactivity of 100 μl of each eluate was measured in 2 ml Quickszint 212 in the scintillation counter. Eluates containing radioactivity were then lyophilized in a Speed Vac concentrator (Savant) coupled to an LO 3 freeze dryer (Wkf). The lyophilisate of each radioactive eluate was dissolved in an appropriate volume of distilled water corresponding to the radioactivity previously determined. Samples (2 μl) of each radioactive lyophilisate were separated by thin layer chromatography (TLC) on silica gel aluminum foils (Merck) in *n*-propanol:distilled water of 3:1 (total volume 60 ml). Autoradiography was conducted by exposing the chromatograms to Fuji RX X-ray film in a cassette with intensifier screen (Sigma) for 7–14 days at a temperature of −45°C. The film was subsequently developed in D19b developer (Kodak) and fixed in Unifix fixer (Kodak) for 5 min in absolute darkness. The film was then kept, for 2 min, in a stop bath containing a small volume of acetic acid in distilled water. Finally the film was intensively washed with water and dried at 30°C.

The experiment to determine the chemical fate of the inducer peptide was performed in replicate. In addition, the following control experiments were carried out:

a) The chemical stability of ^{14}C-dansyl-GPGGPA in ABS was determined by incubating the inducer peptide in 100 μl ABS at a concentration of 1.7×10^{-6} mol/l (0.4 KBq) for 8 h at room temperature. Thereafter the solution was separated by reversed phase chromatography in Octyl Si = 100 in mini columns and further prepared for TLC and autoradiography as described above.

b) As a test of the chemical stability of ^{14}C-dansyl-GPGGPA during homogenization of the buds in 0.1 *M* HCl, the peptide (1.7×10^{-7} mol/l; 0.4 KBq) was added directly to 1000 buds of *Cassiopea andromeda* which were taken up in 1 ml 0.1 *M* HCl. Homogenization in a glass pipette and all further steps up to autoradiographic analysis of the homogenate were carried out as reported above.

c) The chemical stability of ^{14}C-dansyl-GPGGPA in bud homogenate was determined by exposing the radioactively labeled peptide at a concentration of 5.1×10^{-7} mol/l (1.2 KBq) to 1 ml 0.1 *M* HCl containing 1000 homogenized buds of *Cassiopea andromeda* for 8 h at room temperature. Afterwards the homogenate was prepared as described above.

Results

Induction of metamorphosis by ^{14}C-dansyl-GPGGPA

The radioactively labeled inducer peptide ^{14}C-dansyl-GPGGPA triggered metamorphosis of buds of *Cassiopea andromeda*. Buds started to metamorphose when incubated in ABS containing ^{14}C-dansyl-GPGGPA at either test concentration (9×10^{-6} mol/l or 5.3×10^{-5} mol/l). Reversible attachment (stage A, Fig. 1) of the buds to the walls of the glass dishes or to the surface of the solutions took place within 4–6 h. Irreversible attachment (stage B, Fig. 1) was established after 8 h, whereas buds developed up to stage C and D (Fig. 1) when exposed to the inducer for 16–32 h.

Uptake of radioactively labeled peptide by metamorphosing buds

Autoradiographic analysis revealed silver grains in all longitudinal sections of buds, regardless of whether they were exposed to ^{14}C-dansyl-GPGGPA for 10 min or for 32 h (Fig. 2a).

Labeling was discovered in the ectoderm and endoderm. The ectoderm is composed of three cell types: tall columnar cells, nematoblasts and nematocytes, and mucus cells (Hofmann and Honegger, 1990). Silver grains were found in all of these cell types. Accumulation of labeling in a special cell type could not be observed (Fig. 2b). The same was true for the endoderm consisting of columnar cells and cells exhibiting heterolysosomes, digestive

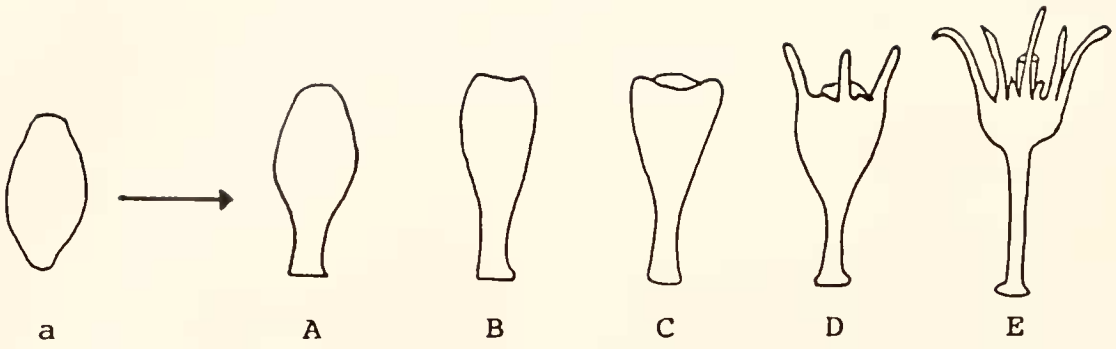

Figure 1. Development of buds (stage a) of *Cassiopea* spp. in the presence of metamorphic inducers. Irreversible attachment takes place at stage B.

vacuoles, and vesicles (Hofmann and Honegger, 1990; Fig. 2b).

Analysis of the silver grain density in sections of buds exposed to ^{14}C-dansyl-GPGGPA for 10 min or 30 min revealed a significant increase in the total number of labels with the duration of the incubation. Comparison of the degree of labeling of the segments for the two incubation times showed that after 30 min the number of silver grains significantly increased in all segments except the head. The ectoderm was found to label more than the endoderm for both 10-min and 30-min incubation (Fig. 3). The density of silver grains was highest in the pedal disc, followed by the stalk and the head. This profile appeared in the 10-min and 30-min specimens (Fig. 3). Comparison of the data of the 10-min incubation showed significant differences in the labeling of the segments in ectoderm and endoderm except for (1) pedal disc endoderm *versus* head ectoderm and (2) stalk endoderm *versus* head endoderm (Fig. 3). This was also true for the 30-min incubation except for (1) stalk ectoderm *versus* pedal disc endoderm and (2) stalk endoderm *versus* head endoderm (Fig. 3).

Chemical fate of ^{14}C-dansyl-GPGGPA

When buds that had been previously exposed to ^{14}C-dansyl-GPGGPA for 3, 8, or 16 h were homogenized in 0.1 *M* HCl, more than 85% of the total radioactivity was

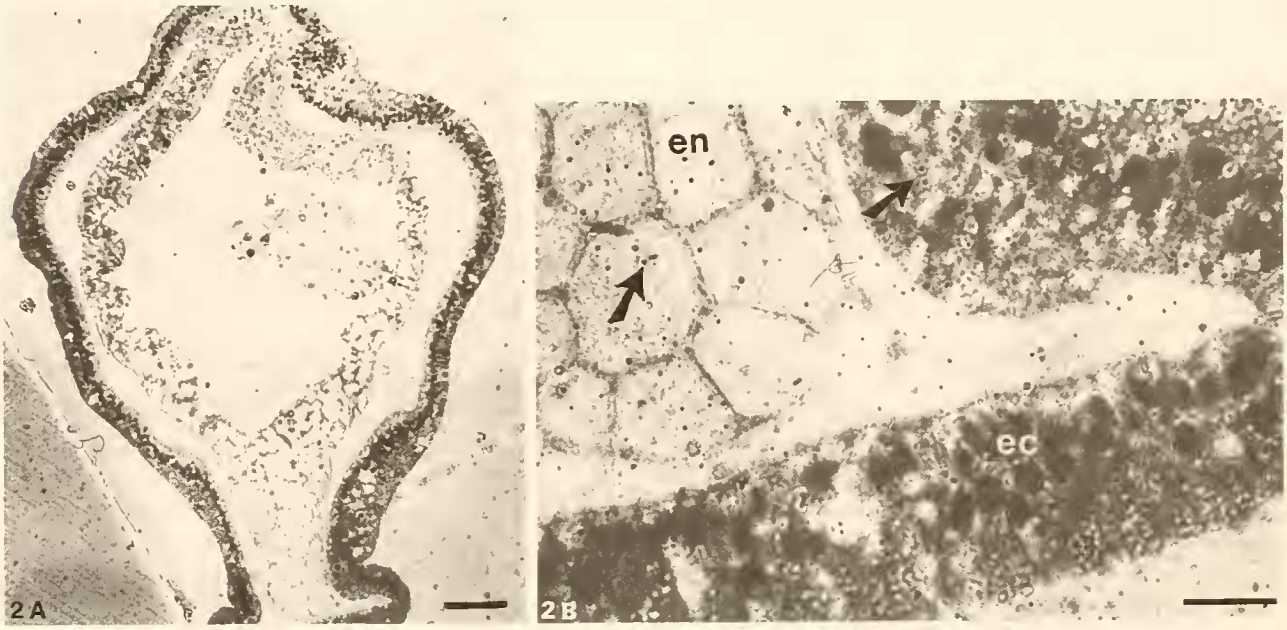

Figure 2. (A) Total view of a longitudinal section through the center of a bud of *Cassiopea andromeda* exposed to ^{14}C-dansyl-GPGGPA (5.3×10^{-5} mol/l) for 30 min. Silver grains are not visible at this magnification (200×). Scale bar, 50 μm. (B) Distribution of silver grains (arrows) in the corresponding pedal disc of (A). en, endoderm; ec, ectoderm. Magnification 1000×; scale bar, 10 μm.

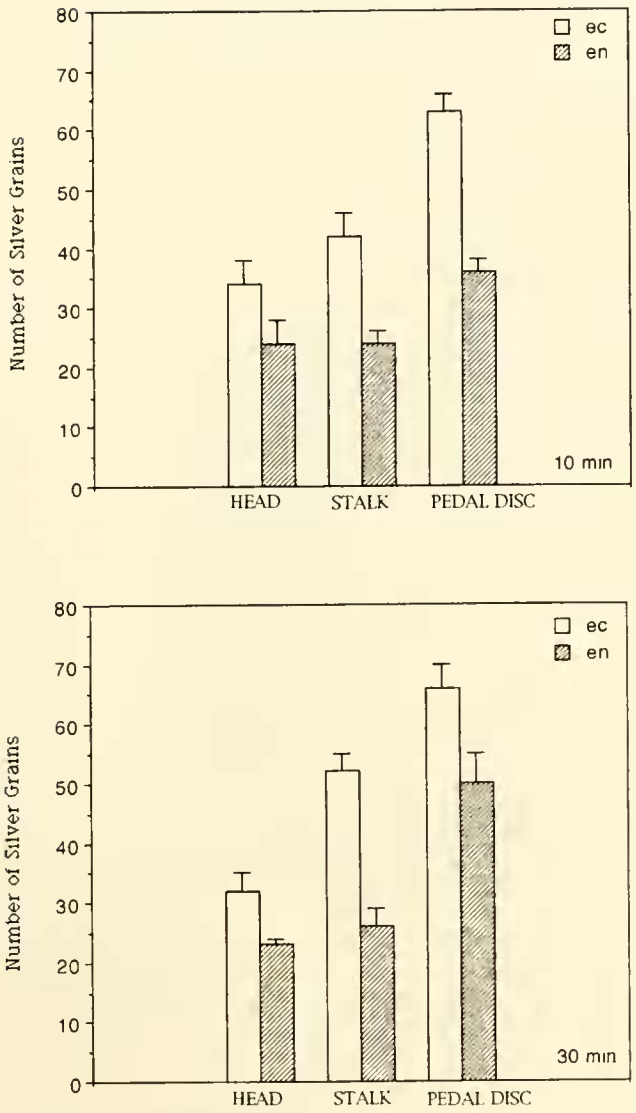

Figure 3. Number of silver grains per cm^2 photo area of longitudinal sections of buds of *Cassiopea andromeda* exposed to ^{14}C-dansyl-GPGGPA (5.3×10^{-5} mol/l; 114 kBq) either for 10 or 30 min. Data are means ± SD of the number of silver grains in 5 sections of one bud. Two-way ANOVAs were carried out for both incubation times, with segment (head, stalk, pedal disc) and tissue (ectoderm, endoderm) as factors. All pairwise multiple comparison procedures were performed with the Student-Newman-Keuls method. ec, ectoderm; en, endoderm.

found in the supernatant after centrifugation of the homogenate. The pellet dissolved in 1.35 *M* NaOH contained less than 14% of the total radioactivity. Therefore, only the supernatant of the homogenate of buds was separated by reversed phase chromatography in mini columns.

Total radioactivity in the 0.1 *M* HCl supernatant increased in a nonlinear manner with the duration of the incubation (Fig. 4). Reversed phase chromatography of the 0.1 *M* HCl supernatant revealed radioactivity in the distilled water eluate and in the 50% methanol eluate (Fig. 4), but at longer incubations, an increasing portion of radioactivity could not be eluted from the reversed phase gel (Fig. 4).

The 50% methanol eluates were lyophilized, redissolved, and subjected to TLC. Autoradiography showed ^{14}C-dansyl-GPGGPA, ^{14}C-dansyl-GP, and ^{14}C-dansyl-G for each incubation time (Fig. 5, lanes B, C, D). The distilled water eluates contained high amounts of salts, and these autoradiograms showed no well-defined spots. Therefore, identification of the radioactive compounds was impossible.

To determine whether degradation products of the hexapeptide occur in the incubation solution in the presence of metamorphosing buds, a 20-μl sample of the incubation solution was analyzed following an 8-h incubation of 1000 buds in 900 μl of ABS containing ^{14}C-dansyl-GPGGPA (9×10^{-6} mol/l; 17.8 KBq). The sample was separated on a mini column by reversed phase chromatography in Octyl Si = 100. Radioactive eluates were checked by TLC followed by autoradiography of the TLC aluminium foil. The autoradiogram showed that traces of ^{14}C-dansyl-GP and ^{14}C-dansyl-G were present in the medium simultaneously with the hexapeptide (Fig. 6, lane A).

The control experiments revealed that (i) ^{14}C-dansyl-GPGGPA was chemically stable in ABS at room temperature for at least 8 h (Fig. 6, lanes B, C, and D); (ii) the chemical stability of ^{14}C-dansyl-GPGGPA was not affected by the homogenization of the buds in 0.1 *M* HCl (data not shown); and (iii) ^{14}C-dansyl-GPGGPA was chemically stable in bud homogenate for an incubation time of at least 8 h (Fig. 6, lanes E and F).

Discussion

The present study, applying the same radioactively labeled inducer to buds of *Cassiopea andromeda* as used for binding experiments *in vivo* (Fleck and Hofmann, 1995), shows that biologically active oligopeptides might face a chemical fate similar to that of many signal molecules. Autoradiography of longitudinal sections of buds incubated in solutions containing ^{14}C-dansyl-GPGGPA revealed uptake of the peptide (Fig. 2). Analysis of homogenates of buds that had been exposed to the hexapeptide for 3, 8, or 16 h showed that the inducer was partly degraded to ^{14}C-dansyl-GP and ^{14}C-dansyl-G (Fig. 5). Those cleavage products are not able to induce metamorphosis (Fleck, 1994). Internalization and degradation may therefore represent a mechanism for signal termination.

Uptake of peptidic signal molecules is common in plant and animal cells. This process may lead to intracellular degradation of the ligand and receptor but often involves recycling of the receptor to the cell surface (see review by Smythe and Warren, 1991). In the present study, radioac-

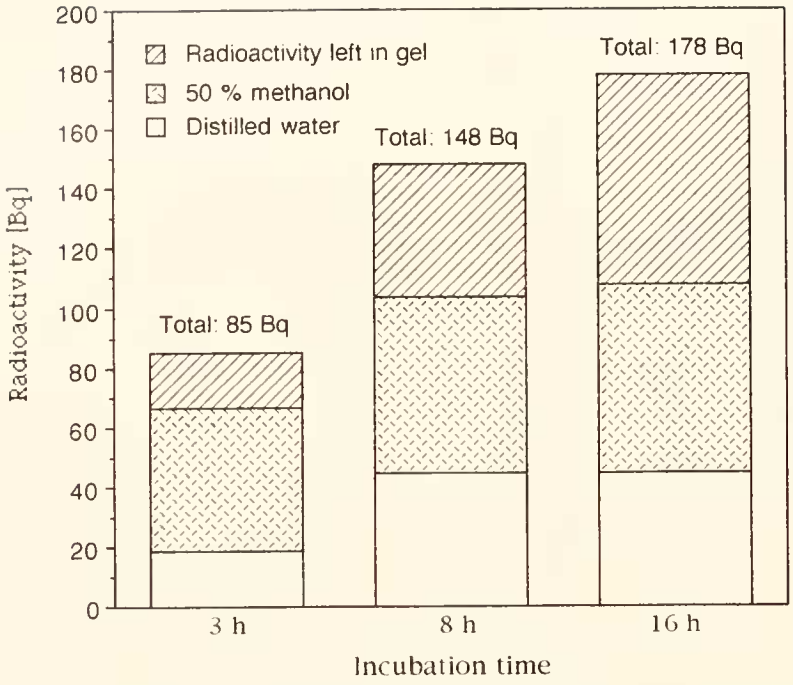

Figure 4. Radioactive fractions resulting from reversed phase chromatography of the supernatants of homogenates of buds of *Cassiopea andromeda* following 3, 8, or 16 h incubation with ^{14}C-dansyl-GPGGPA (9×10^{-6} mol/l; 17.8 kBq). Data are representative results of one out of two chromatographic separations.

tively labeled peptide was shown to be internalized rapidly by buds of *Cassiopea andromeda* when exposed to the metamorphosis-inducing oligopeptide ^{14}C-dansyl-GPGGPA. Silver grains were discovered in longitudinal sections of buds that had been incubated with the inducer for only 10 min. Counting of the labels and relating their number to the three morphological parts of the presumptive polyp (pedal disc, stalk, head) revealed that labeling was highest in the ectoderm of the pedal disc. An explanation for this degree of labeling may lie in the fact that buds and larvae swim with the aboral knob—which becomes the pedal disc upon metamorphic induction—facing forward when exploring their habitat in search of a substrate suitable for settlement. In addition, receptors for inducer peptides are also supposed to be concentrated at the aboral end of buds and larvae (Fleck and Hofmann, 1995).

Silver grains were not found to be concentrated in specific cells, which may indicate that all cell types are able to take up peptide. The uptake mechanism may be specific, nonspecific, or both.

Which chemical molecule is represented by the silver grains in the longitudinal sections? The supernatant of homogenized buds given a prior exposure to ^{14}C-dansyl-GPGGPA for 3, 8, or 16 h showed that the degradation products ^{14}C-dansyl-GP and ^{14}C-dansyl-G were present in addition to the original compound (Fig. 5). The total amount of these agents increased with the duration of the incubation. Although longitudinal sections of buds incubated with the inducer for only 10 min or 30 min were analyzed for silver grain density, cleavage products of the hexapeptide might have already occurred after these short exposures. Silver grains would then represent not only ^{14}C-dansyl-GPGGPA but also ^{14}C-dansyl-GP and ^{14}C-dansyl-G.

Two radioactive fractions isolated from bud homogenate could not be identified. The analysis of one of these fractions was impossible due to the high content of salt in the sample. The other portion of radioactivity, which increased with the duration of the incubation, could not be eluted from the gel. Compounds larger than 20 kD or very hydrophobic molecules usually cannot be eluted from the type of reversed phase gel used in this study (pers. commun., Boehringer Biochemicals, Mannheim). Therefore, the noneluable substances might reflect hexapeptide bound to large compounds such as proteins and lipids, or denatured hexapeptide. However, silver grains in the longitudinal sections may even represent products of these unidentified radioactive fractions.

It is remarkable that ^{14}C-dansyl-GP and ^{14}C-dansyl-G are not able to effect metamorphosis (Fleck, 1994). Carr *et al.* (1990) reported that degradative enzymes in chemosensory processes can change chemoactive compounds into ones that are less or more stimulatory. Signal termination can be obtained by this mechanism in organisms

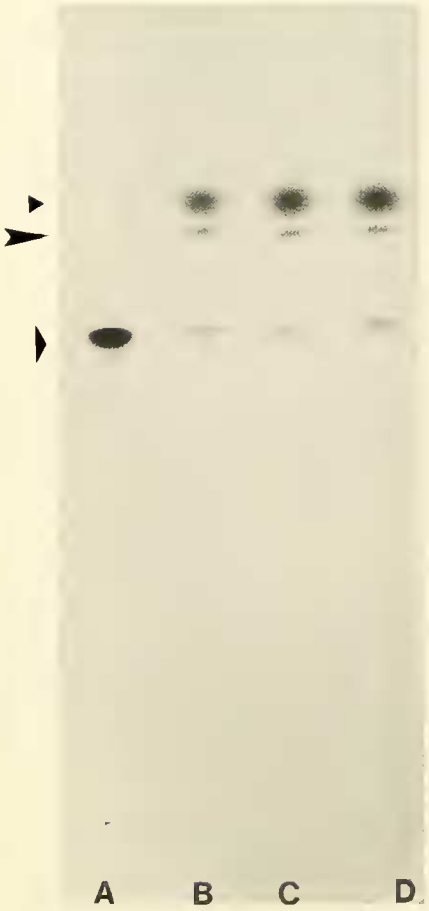

Figure 5. Autoradiogram of the radioactive 50% methanol eluate obtained by reversed phase chromatography of the supernatant of homogenates of buds of *Cassiopea andromeda* exposed to ^{14}C-dansyl-GPGGPA (9×10^{-6} mol/l) for 3 h (lane B), 8 h (lane C), or 16 h (lane D). A standard of ^{14}C-dansyl-GPGGPA was applied in lane A. ◗, ^{14}C-dansyl-GPGGPA; ➤, ^{14}C-dansyl-GP; ▶, ^{14}C-dansyl-G.

including slime molds, yeast, insects, and crustaceans (Carr *et al.*, 1990). The spiny lobster *Panulirus argus* detects potential food sources by olfactory sensilla on the antennules. These sensilla contain receptors that are sensitive to exogenous nucleotides. In addition, the sensilla contain membrane-bound degradative enzymes that rapidly dephosphorylate nucleotides entering the sensillar lymph and pumps that internalize different biologically active molecules. This process results in a change of the stimulatory state of the signal (Trapido-Rosenthal *et al.*, 1987, 1989; Gleeson *et al.*, 1991, 1992).

Such degradative enzymes seem also to be present in buds of *Cassiopea andromeda*. Action of these enzymes should have degraded ^{14}C-dansyl-GPGGPA to the biologically inactive compounds ^{14}C-dansyl-GP and ^{14}C-dansyl-G. However, the experiments performed in this study did not provide any information about the chemical fate of the carboxyterminal cleavage fragments of the inducer peptide. Therefore the possibility cannot be excluded that, in addition to the products that did not evoke metamorphosis, peptidic fragments were produced (*e.g.*, GGPA) that are able to induce metamorphosis even though they are less stimulatory than ^{14}C-dansyl-GPGGPA (Hofmann *et al.*, 1996).

Degradation of the hexapeptide may take place either externally or internally of the buds, or by a combination of both processes. On the one hand, enzymes that are either membrane-bound or released into the medium may hydrolyze the inducer right after binding to receptors located in the cell membrane. Cleavage products can then be internalized by the metamorphosing buds. The presence of ^{14}C-dansyl-GP and ^{14}C-dansyl-G in the incubation

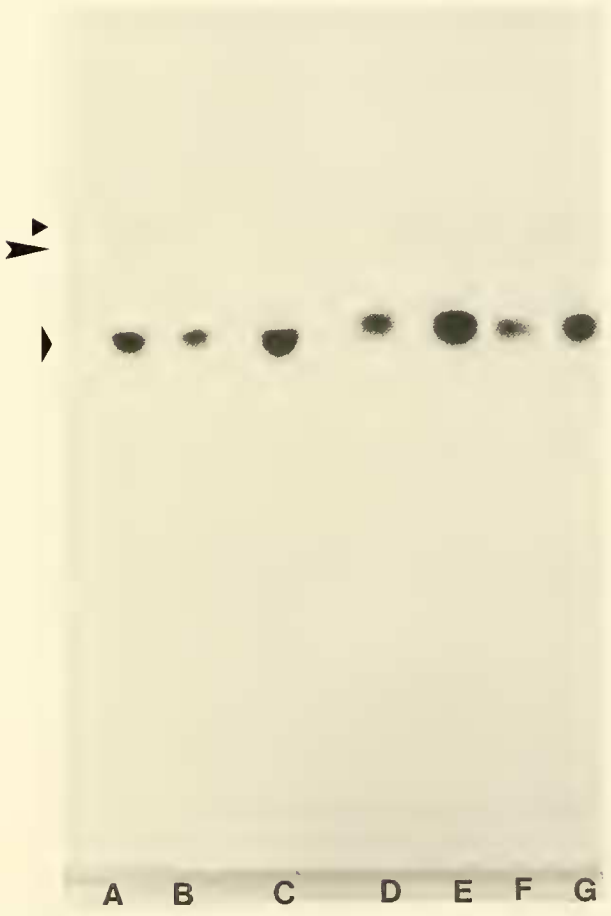

Figure 6. Autoradiogram of radioactive eluates obtained by reversed phase chromatography of the following solutions. Lane A: sample of the incubation solution of 1000 buds of *Cassiopea andromeda* exposed to ^{14}C-dansyl-GPGGPA (9×10^{-6} mol/l) for 8 h. Lanes B, C, D: ^{14}C-dansyl-GPGGPA incubated in ABS for 8 h at room temperature. Lanes E, F: ^{14}C-dansyl-GPGGPA incubated in a homogenate of 1000 buds of *Cassiopea andromeda* for 8 h at room temperature. Lane G: standard of ^{14}C-dansyl-GPGGPA. ◗, ^{14}C-dansyl-GPGGPA; ➤, ^{14}C-dansyl-GP; ▶, ^{14}C-dansyl-G.

solution after 8 h may support this hypothesis. This inactivation mechanism would reduce receptor desensitization and allow receptor activation to be renewed by subsequent pulses of the inducer peptide (Carr *et al.*, 1990). Gleeson *et al.* (1991, 1992) showed that degradative enzymatic activity in *Panulirus argus* is located in the transitional zone of the olfactory sensilla, which is the region in which the sensory neurons develop cilia and branch to form the outer dendritic segments. This demonstration might suggest that peptide-cleaving enzymes of *Cassiopea* spp. are also membrane-bound rather than released into the extracellular medium.

On the other hand, hexapeptide bound to its receptor may be taken up as a ligand-receptor complex. Degradation of the inducer and recycling of the receptor to the cell membrane as described for the epidermal growth factor (see review by Sorkin and Waters, 1993) would follow the internalization process. The presence of ^{14}C-dansyl-GP and ^{14}C-dansyl-G in the 8-h incubation solution would then indicate that buds released cleavage products of the original signal molecule. This inactivation mechanism would also allow renewal of receptor activation since a certain number of surface receptors would be re-exposed in the cell membrane after recycling. In addition, nonspecific uptake of peptide may occur during both processes described above.

Limited desensitization of receptors could be of special importance for metamorphic induction of *Cassiopea* spp. triggered by peptides. ^{14}C-dansyl-GPGGPA used in a concentration of 9×10^{-6} mol/l had to be present for 8 h to induce irreversible metamorphosis of buds in this study. Hofmann and Brand (1987) had to apply the inducer peptide Z-GPGGPA for 7 h at 1.2×10^{-5} mol/l to yield 100% metamorphosis of buds of *Cassiopea andromeda* within 24 h. However, buds treated eight times for 1 h with the same concentration of the hexapeptide also underwent metamorphosis to 100% (Hofmann and Brand, 1987). These results suggest that renewed activation of a receptor may be important for irreversible attachment and metamorphosis of buds and larvae of *Cassiopea* spp.

The radioactivity of the fraction that contained ^{14}C-dansyl-GPGGPA, ^{14}C-dansyl-GP, and ^{14}C-dansyl-G still increased after an incubation time of 8 h. Since receptor activation should no longer be required to complete metamorphosis after this time, the increase found after 16 h might have been the result of nonspecific interactions.

The findings reported here are in contrast to results published by Trapido-Rosenthal and Morse (1986) for the chemical fate of a metamorphic inducer of larvae of the mollusc *Haliotis rufescens*. These authors found that ^{3}H-baclofen, which mimics the inducing capacity of GABA as a settlement cue, was not chemically altered during interaction with the larvae.

The present paper provides data showing that a peptidic metamorphic inducer is chemically changed when exposed to vegetative buds of *Cassiopea andromeda*. The demonstration of the existence of biologically inactive fractions resulting from cleavage of ^{14}C-dansyl-GPGGPA supports the hypothesis that signal termination may be the cause of degradation of the original signal molecule.

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